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나병환자에 있어서 사이토카인의 생산

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하 대 유

- Abstract -

Cytokine Production in Patients With Leprosy

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나병은 세포성 내재균인 나균에 의해서 발생하는 만성 전염병이다. 아직도 나균이 순수 배양 되지 않고, 나균-특이성 백신도 개발되지 않았지만 전 세계적으로 나병의 발생은 세계보건기구(WHO)가 주도한 복합나화학요법(MDT) 프로그램과 정부 및 비정부의 기관의 노력으로 현저하게 감소되고 있다. 그러나 과거 20년에 등록된 환자수가 감소되고 그리고 개인의 나병 감염은 치료가 가능함에도 불구하고 등록한 신환자의 수는 크게 변화되지 않고 있다고 한다. 세계적인 나병 퇴치는 많은 노력에도 불구하고 아직은 성취되지 못하고 있다. 나병은 치료과정에서 부작용으로 상당히 빈번히 발생하는 염증반응, 즉 가역성반응(I형 반응)과 나성결절성홍반(II형 반응)이 발생한다. 그러나 이들 나반응(leprosy reaction)은 그 발생원인, 발생기전, 그 치료방법과 면역기전은 아직도 확실히 알지 못한다. 각기 다른 형의 나반응은 각기 다른 면역기전에 따라 발생한 것 같지만 아직도 정확한 기전은 잘 알려져 있지 않다.

최근에 몇 가지 작동 T세포가 발견됨으로서 협조 T(Th)세포 연구에 박차를 가하고 있다. 성숙한 작동 T세포는 새로운 여러 가지 사이토카인(cytokine)을 생산하고, 생성된 사이토카인은 면역반응을 조절하는 여러 가지 기능을 가지고 있기 때문에 최근 활발한 연구대상이 되고 있다. 최근에 새로운 Th세포가 발견되었다. 이들 Th1, Th2, Th7, Th9, 여포협조 T (Thf)세포 그리고 면역조절 T(Treg)세포는 각기 여러 가지 사이토카인을 생산한다. 이들 세포는 림포카인, 염증촉진 사이토카인, 항염증 사이토카인, 성장인자, 그리고

케모카인(chemokine) 등을 생산한다. 이 사이토카인은 실로 여러 가지 중요한 생물학적 과정, 즉, 줄기세포 증식, 림프구의 분화, 세포 진화, 면역반응 등에 관여하기 때문에 암, 바이러스 감염 등 여러 가지 질병치료에 임상적으로 사용 될 뿐만 아니라 질병의 예방, 진단, 예후 등에도 광범위하게 사용되고 있다는 사실은 놀랄 일이 아니다.

저자는 이 종설에서 나병에 있어서 사이토카인의 역할, 나반응의 형에 따른 사이토카인의 프로필, 항-나병치료가 사이토카인 생산에 미치는 영향에 관하여 논하고, 실험적 나병 감염 그리고 I형 및 II형 나반응 동안에 사이토카인 유전자발현의 변화에 관하여도 논할 것이다. 또한, 나병의 면역병리에 관하여 아직도 해결되지 않은 문제에 관하여 간단히 논할 것이다.

Key Words : Chemokine, Chemokine Receptor, Cytokine, Effector T cells, Leprosy Reaction.

INTRODUCTION

Mycobacterium leprae is the etiologic agent of leprosy, an infectious, neurodegenerative human disease. Leprosy and its associated disabilities are unlikely to disappear soon, as recognized in the World Health Organization's (WHO's) Global Strategy 2006~2010. Despite a spectacular decrease in global prevalence of leprosy since 1982 to 219,826 cases in 2005, the incidence appears not to have changed significantly, and in fact, leprosy is more common than expected[1], with 296,499 new cases in 2005 (158,728 multibacillary, 90,122 females, 28,005 children, 13,361 cases of grade 2 disability) (http://www.who.int/wer).

The continued incidence of leprosy in countries where it is endemic is thought to result from the perpetuating reservoir of *M. leprae*-infected contacts or persons with subclinical leprosy[2]. It is generally held that dissemination of *M. leprae* is from nasal mucosa and not through the skin of infected patients. Importantly, however, Job et al recently reported that both skin and nasal epithelia of untreated multiple bacillary leprosy patients contribute to the shedding

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of *M. leprae* into the environment and contacts of untreated cases are at risk of contact with *M. leprae* through both the nasal mucosa and exposed surfaces of their skin[3]. Leprosy is not going to disappear anytime soon. Effective multidrug regimens are now used worldwide, and the infection in individuals is curable. However, although the reported number of registered cases worldwide has declined in the last two decades, the reported number of new cases registered each year has remained the same (at 500,000 to 700,000) over the same interval[4]. A great deal of important new information has been generated by recent research. Authoritative overviews on progress in leprosy have been published in recent years[2-13]. Leprosy is a chronic infectious disease of the skin and nerves caused by the intracellular pathogen *M. leprae*. It is characterized by a spectrum of clinical forms depending on the host's immune response to *M. leprae*[14]. Patients with tuberculoid leprosy (TT) have strong cell-mediated immunity (CMI) with elimination of the bacilli, whereas patients with lepromatous leprosy (LL) exhibits defective CMI to *M. leprae*. Between these two polar forms of disease are indeterminated (I) and the unstable borderline forms, including mid-borderline (BB) borderline tuberculoid (BT), borderline lepromatous (BL) on the basis of dermatological, neurological and histopathological findings[15]. WHO established a more simplified classification system which consists of just two categories-paucibacillary (PB) and multiple bacillary (MB). (WHO Expert Committee on Leprosy 1988, Seventh report, World Health Organiztion, Geneva). PB leprosy is defined as five or fewer skin lesions with no bacilli in skin smear positive.

Leprosy may be complicated by inflammatory reactions. During the clinical course of leprosy, a substantial proportion of patients develop leprosy reactions. Reactions are acute inflammatory complications often presenting as medical emergencies during the course of treated or untreated Hansen's disease. Two major clinical types of leprosy reactions occur: together they may affect 30 to 50% of all leprosy patients[16-18]. Because *M. leprae* infects peripheral nerve, the inflammation associated with reactions is a medical emergency, as severe nerve injury may develop rapidly, with subsequent loss of sensation, paralysis, and deformity. The cause, mechanisms, and treatment of these reaction remain highly problematic, for both clinicians and basic scientists. The different types of reactions appear to have different underlying immunologic mechanisms, but these are poorly understood in spite of a substantial body of detailed descriptive information, and the factors that initiate them

are unknown[19]. Type 1 reactions (T1R) occur in patients in the borderline portion of the spectrum (BL, BB and BT). They are also known as reversal reaction (RR), because after the reaction had subsided, clinical and histopathological evidence indicated that the immunity in the lesions had increased or decreased[14]. *M. leprae* antigens have been demonstrated in the nerve and skin of patients experiencing T1R[9]. Type 2 reactions, also known as erythema nodosum leprosum (ENL) occur in multibacillary patients (LL an BL). These patients experience an abrupt onset of crops of very tender, erythematous nodule that may develop on the face, extremities, or trunk without predilection for existing lesions. Systematically, these patients often also experience fever, malaise and some degree of neuritis[9].

Cytokines are low-MW protein mediators that usually act at short range between neighboring cells, have been studied extensively for the past 30 years. These molecules, previously also termed interleukin (IL), interferon (IFN), growth factors, and tumor necrosis factor (TNF), among other designations, are involved in essentially every important biological process, from cell proliferation to inflammation, immunity, migration, fibrosis, repair, and angiogenesis[20]. As these molecules and their associated receptors provide key signals for important processes, it is not surprising that abnormalities in cytokines, their receptors, and the signaling pathways that they initiate are involved in a wide variety of diseases. Indeed, they have a role in far too many diseases. Rather, representative examples of the cytokines involved in leprosy are discussed. Over 100 cytokines have now been identified and are classified into lymphokines (cytokines that are secreted by T cells and regulate immune response, proinflammatory cytokines (cytokines that amplify and perpetuate the inflammatory process), antiinflammatory cytokines (cytokines that negatively modulate the inflammatory response), growth factors (cytokines that promote cell survival and result in structural changes in the airways) and chemokines (cytokines that are chemotactic for inflammatory cells, although many of these functions may overlap. The critical issue in each case is the importance of cytokines as therapeutic targets, and the overwhelming message is that "anti-cytokine medicine" is a rapidly growing field with major pharmaceutical impact[20]. Proinflammatory and anti-inflammatory cytokines and their signaling pathways play key roles in protection from and pathogenesis of mycobacterial infection. Cytokines produced by innate and adaptative immunity have been demonstrated to play an active role in protection against

intracellular pathogens that regulate the interaction of immunocompetent cells and act as effectors of antimicrobial immunity[20,21]. The classically described Th1 and Th2 cells and the cytokines they produce have been demonstrated, in murine models of bacterial and parasitic diseases, to be pivotal for protection or immunopathology[22,23]. The two fundamental arms of the immune response, innate and adaptive immunity, jointly form a defensive front against pathogens. Effector T cells are the key players in steering the immune responses to execute immune functions. The differentiation of naive T cells into fully functional effector T cells is characterized by the acquisition of new profile of cytokine production and is largely directed by cytokines produced by activated cells of the innate, as well as adaptive, immune system. Key advances in the understanding of effector T cells have been tied to cytokine biology. Cytokine production is vital for the classification and function of effector T cells. Owing to their critical and diverse functions in controlling immune responses, the functions and regulation of cytokines have been a subject of intense investigation since the Th1 and Th2 paradigm was conceived and established in 1986[22]. A seminal study by Coffman et al revealed that cytokine profiles can be used to categorized CD4 effector cells, namely helper-T-cell-1 and -2 (Th1 and Th2), which possess different immunological functions[20,22,24]. Recently, several types of effector T cells have been documented with distinct biological functions. Th1, Th2, Th17, Th9 and follicular helper T (Tfh) cells are involved in inflammatory responses[25] while regulatory T (Treg) cells engage in immune suppression[23,26,27] (Table 1).

Table 1. Production of cytokines from effector CD4 helper T cells (Th)

CD4 effector T cell subsets	Cytokine production	Effector functions (Pathogen cleared)
Th1	IL2, IL-12, IL-18, IL-27, IFN-γ, TNF-α, TNF-β (lymphotoxin)	Intracellular pathogens, e.g. *M. leprae*
Th2	IL-4, IL-5, IL-6, IL-9, IL-10, IL-13	Extracellular pathogens
Th9	IL-9, IL-10	Helminthes
Th17	IL-17, IL-21.IL-22, IL-23, TGF-β	Extracelluar pathogens
Tfh	IL-6 .IL-10, IL-21	Antibody formation in lymph node follicles
Treg	IL-10, IL-2, TGF-β, TSLP*	Immunosuppression, anti-inflammatory

CD4 effector T cells are defined based on the cytokines produced. Th1, Th2 Th9, Th17 and follicular helper T cells (Tfh) are involved in inflammatory responses while regulatory T cells (Treg) engage in immune suppression. Th cells contributed to the clearance of specific types of pathogens. Recently, Th17 and Th9 subsets have been identified and these cells induce experimental autoimmune encephalomyeliis[3-7]. * TSLP : thymic stromal lymphopoietin

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As described above, cytokines are large family of more than 100 small proteins and are involved in essentially every important biological process, from cell inflammation to immune responses, cell growth, cell migration, fibrosis, and angiogenesis, so it is likely that every disease will involve multiple cytokines. As these molecules and their associated receptors provide key signals for important process, it is not surprising that abnormalities in cytokines, their receptors, and the signalling pathways that they initiate are involved in a wide variety of disease. Indeed, they have a role in far too many disease. Cytokines produced by innate and adaptative immunity have been demonstrated to play an active role in protection against intracellular pathogens that regulate the interaction of immunocompetent cells and act as effectors of antimicrobial immunity[24,28]. As shown in Table 1, Th1 cells produce IL-2, IL-12, IL-18, IL-27, IFN-γ, TNF-α, TNF-β[24,29]. Th2 cells produce IL-4, IL-5, IL-6,IL-10, IL-13. Th9 cells produce IL-9 and IL-10[30]. Th17 cells produce IL-17, IL-21, IL-22, IL-23, TGF-β[31]. Tfh cells produce IL-6, IL-10, IL-21[25]. Treg cells produce IL-2, IL-10, TGF-β, and thymic stromal lymphopoietin (TSLP)[23,26,27,30]. These cells interact with cross-regulation producing a variety of cytokine. Resistance to *M. leprae* is mediated through cellular immune response and involves a complex interplay of cytokines and chemokines. Extensive intercellular communication, chiefly through cytokine and chemokine signals, is required to orchestrate cellular accumulation and results in a three-dimensional structure that limits or prevents dissemination of the pathogen and is largely protective[12].

To date, accumulating data has been providing evidence that cytokines play an important role in the immune response to *M. leprae* and in pathogenesis of leprosy [9,11,12,25,32-35]. In this Review, I will briefly outline the evidence describing roles of cytokine in leprosy. I will also discuss cytokine profiles in different types of leprosy reactions and effect of anti-leprosy drug treatment on cytokine

production. And, I will review the recent studies demonstrating cytokine gene expression changes during type 1 and type 2 leprosy reactions.

CYTOKINE IN LEPROSY

Little information exists regarding the levels of specific cytokines in patients with leprosy. In patients with nontuberculous mycobacterial lung disease, production of IFN-γ, TNF-α and IL-12 is significantly lower than those of the healthy controls [36]. IL-17-and IL-22-producing CD4+ T cells may play important roles in the human immune response to mycobacteria[8]. Early pioneering works revealed an apparent relationship between the dominant cytokine profile and the clinical presentation of leprosy. Sasaki et al have reported that IL-2 and IFN-γ were markedly dominant in tuberculoid lesions, whereas IL-4, IL-5 and IL-10 were characteristic of lepromatous lesions[15]. Th1-Th2 dichotomy is a central determinant of type of host defense, namely the Th1 subset characterized by predominant IL-1 and IFN-γ preferentially elicits CMI, whereas Th2 cell which produce IL-4, IL-5 and IL-10 augment humor immunity. Both the classic reciprocal relation between antibody production and CMI and resistance or susceptibility to the leprosy bacillus can be explained by T cell subsets differing in patterns of cytokine production. A study demonstrated that endogenous IL-4 production inhibited IL-10 secretion and, concomitantly, increased TNF-α and GM-CS release. The data suggest that, on balance, IL-4 and IL-10 contribute to immunosuppression in human leprosy infection[37]. Lepromatous disease is characterized by poor granuloma formation. mRNA production is predominantly for cytokines IL-4, IL-5 and IL-10[9]. IL-4 has been shown to down regulate Toll-like receptor-2 (TLR2) on monocytes, and IL-10 will suppress production of IL-12. This is associated with a preponderance of CD8+ lymphocytes in lepromatous lesions[9]. Type 1 CD4 cells reactive with lepromin and PPD produce IFN-γ and IL-2 predominantly. Suppressor T cell clones derived from immunologically unresponsive lepromatous leprosy patients are antigen-specific, CD8 cells, HLA-DQ restricted, and produce predominantly IL-4, and were designated Type 2 CD8 cells[38].

Granulomas in biopsies of untreated patients with tuberculoid leprosy showed large amount of cells positive for IL-1β TNF-α, IFN-γ, whereas no positive signals could be detected in untreated patients with lepromatous leprosy[39]. Remarkably, in tuberculoid

leprosy patients, the number of IL-1 β-positive cells did not vary under therapy, while the number of TNF-α and IFN-γ reactive cells decreased. T cell lines from healthy individuals and TT patients responding to *M. leprae* produced high levels of IFN-γ and TNF-α but little or no IL-4 and IL-6[40]. At the lepromatouse pole, T cell lines failed to proliferate upon stimulation with *M. leprae* but in some case produced significant levels of IFN-γ. No IL-4 or IL-6 secretion was observed in response to *M. leprae*[40]. Sieling et al have found that tuberculoid leprosy lesions have a predominance of CD4+ T cells producing the type 1 cytokine pattern[41]. Furthermore, they found that IL-12 mRNA was expressed at 10-fold higher levels in tuberculoid lesions as compare to lepromatous lesion and that IL-12 promotes the selective expansion of the type 1 cytokine producing cells. In addition, IL-10 also expressed at higher levels in lepromatous as compared to tuberculoid lesions, was found to be produced by macrophages, effectively inhibiting cytokine production and macrophage activity[41]. Overall, many studies have generally revealed a predominance of IL-2, TNF-α, and IFN-γ transcripts in tuberculoid lesions and IL-4 and IFN-γ in lepromatous ones, gene expression profiles consistent with Th-1 and Th-2 patterns, respectively[39-43]. CD4+ clones isolated from TT lesions secreted primarily IFN-γ, whereas a CD4+ clone from an LL lesion produced predominantly IL-4[44], and CD8+ clones isolated from LL patients likewise generated large amounts of IL-4[45]. Further studies have also indicated that IL-12 and IL-18 promote resistance to *M. leprae* and are highly expressed in tuberculoid lesions[41,46]. The studies noted above have examined biopsies from well-established lesions, usually present for at least 2 years. The immunologic activity within earlier lesions, in patients with only a single lesion, has recently been evaluated by Stefani et al[47]. These early lesions, histologically consistent with TT or BT disease, also displayed a Th-1-like pattern of cytokine gene expression.

Elucidating the pathways that regulate cytokine production remains a central focus toward understanding immune responses in human infectious disease. Yamauchi et al investigated to address the role of the CD40-CD40L pathway in generating IL-12 production in human infectious disease, using leprosy as a model[48]. They presented evidence that the level of CD40 and CD40L expression in leprosy correlates with CMI to the pathogen. In particular,

CD40-CD40L interactions contribute to CMI responses in the tuberculoid form of leprosy by inducing IL-12 production in monocytes. They also show that such interactions are not evident in lepromatous leprosy patients. CD40 is a 50-kDa glycoprotein present on the surface of B cells, monocytes, dendritic cells, eosinophils and endothelial cells and is a member of the TNF receptor superfamily. The ligand for CD40 (CD40L) is induced on the surface of T cells after activation[48]. Increasing evidence has supported the idea that CD40-CD40L interactions are critical for T cell-dependent activation of monocytes. Ligation of CD40 stimulates macrophages to produce a variety of cytokines, including IL-12[48]. IL-12 is a key regulator of Th1 cytokine responses in vitro as well as in mouse models of infection[45]. The action of IL-12 is mediated through an IL-12R composed of ß1 and ß2 subunits: the latter is selectively expressed primarily on type 1 cytokine-producing T cells[43,47,49]. Furthermore, patients with mutations in their IL-12R have increased susceptibility to mycobacterial infection [43,47,49]. Verhagen et al generated a large number of *M. leprae*-responsive and *M. leprae*-nonresponsive T cell clones (TCC) from the lesional skin of immunologic unstable borderline leprosy patients and investigated the secretion of a panel of other cytokines (TNF-α, IL-5, IL-6, Il-10 and IL-13) by a large number of these TCC[50]. They observed a positive correlation in the coproduction of IFN-γ/TNF-α, and in that of IL-4/IL-5, IL-4/IL-13 and IL-5/IL-13. Polarized type1-like TCC produced dominant IFN-γ/TNF-α, and polarized type 2-like TCC predominantly IL-4/IL-5/IL-13. These results suggested that distinct isotypes of type 1 and type 2-like T cells, based on the secretion of a panel of cytokines may reflect *M. leprae*-specific characteristics. Patients with tuberculoid leprosy are able to restrict the growth of the pathogen and their skin lesions are characterized by a predominance of CD4+ T cells and type 1 cytokines including IL-2 and IFN-γ. At the opposite pole, patients with lepromatous leprosy are unable to contain the infection and their skin lesions are characterized by a predominance of CD8+ T cells and type 2 cytokines including IL-4 and IL-10. A key determinant of the T-cell cytokine response may be IL-12, which selectively favors expansion of CD4+ T cells producing IFN-γ[51]. These divergent cytokine patterns are regulated in part by the local production of IL-12, which is present in 10-fold greater

levels in lesions from tuberculoid than in those from lepromatous patients[41]. IL-12 can contribute to host resistance in human infectious disease by inducing the selective expansion of type 1 cytokine-producing T cells. In turn, several factors contribute to the regulation of IL-12 production. One determinant of IL-12 production is IFN-γ, which in leprosy patients up-regulates IL-12 production and down-regulates the inhibitory cytokine, IL-10[44].

The more benign PB forms of leprosy BT and TT leprosy are characterized by the predominance of a Th1-type immune response, the presence of well-formed granulomas at the site of the lesion, and control of mycobacterial replication[52]. In contrast, in the MB forms BB and BL leprosy and LL leprosy no granuloma is seen, and high bacterial load and antibody levels are detected. Cytokines evidently play a critical role in triggering host-pathogen interactions. On one hand, greater TNF-α production, indicated by the presence of TNF-α mRNA in blood mononuclear cells, has been described in patients with BT or TT leprosy than in patients with LL. This suggests that TNF-α has a role in protection against the severe forms of the disease[53]. On the other hand, it has been suggested that IL-10 plays a role in the induction and/or maintenance of anergy in patients with LL[54]. These data indicate that a relationship exists between TNF-α and IL-10 promoter polymorphisms and the development of PB leprosy[52].

The outcome of infection in murine models of infection and in human infectious disease is regulated by the Th1 and Th2 T cell cytokine patterns. Th1 cells secrete IL-2 and IFN-γ and are generally associated with resistance to intracellular pathogens, whereas Th2 cells secrete IL-4 and IL-10 and are associated with progressive disease to these same pathogens. It has become increasingly evident that IL-12 is a pivotal regulator of Th1 responses and is essential for promoting CMI in host defense in leprosy[44,51]. A study showed that lepromatous patients with disseminated disease had Th2-type cytokines, with IL-4 but not IFN-gamma[54]. In contrast, tuberculoid leprosy patients with localized disease showed a Th1-like profile, with the presence of IFN-γ, but not IL-4. Circulating leukocytes and T-cell lines from tuberculoid patients stimulated by *M. leprae* in vitro have also generally been found to produce a Th-1 cytokine pattern, while leukocytes and T-cell

lines from lepromatous patients generally produce a Th-2 cytokine pattern[54,55]. However, leukocytes from approximately 40% of all patients in one such study produced a mixed Th-0 cytokine profile, i.e., IFN-γ, IL-2, and IL-4[54]. It is possible that some of the patients whose cells produced the Th-0 pattern were in the borderline portion of the leprosy spectrum (BL or BT): alternatively, the human immune response to *M. leprae* may not correspond entirely with the Th-1/Th-2 model. Fractionated *M. leprae* antigens have also been found to stimulate IFN-γ in vitro with leukocytes from tuberculoid patients[6]. Moraes et al showed that cytokines such as IL-6 IL-8, TNF-α and TNF-β were present in reactional and tuberculoid patients as opposed to BL/LL patients[53]. Interestingly, the majority of ENL/RR patients showed message for IL-6, IL-10, IL-12 and TNF-α in skin. IFN-γ was detected in 84.6% (ENL) and 100% (RR) of the patients, whereas IL-4 was detected only in few individuals (38.5% and 25%, respectively).

Experimental immunotherapy with intradermal inoculation of cytokines has provided additional information about their roles in immunological events within leprosy skin lesions[57]. Both short and long-term intradermal administration of IFN-γ resulted in an influx of mononuclear cells and an increase in the CD4/CD8 ratio in the lesions, but did not reverse the specific nonresponsiveness of circulating leukocytes to *M. leprae*[58]. *M. leprae* exposure in vitro did not elicit IFN-γ in circulating mononuclear cells of lepromatous patients: the addition of IL-2 reversed this in most of these patients' peripheral blood mononuclear cells(PBMCs)[59]. In lepromatous patients, intradermal injections of IL-2 generated apparent increases in CMI within the skin lesions[60]. And, the administration of rIL-2 has had a significant effect in decreasing the total body burden of *M. leprae*[61]. The shift to the production of a Th1 cytokine profile during an intracellular infection has been shown to depend on antigen presenting cells-derived IL-12 and T-cell-derived IFN-γ production. IL-18 facilitated Th1 priming in synergy with IL-12 through the stimulation of IFN-γ producion by T cells, B cells, NK cells, macrophages and dendritic cells[34]. They found that IFN-γ levels in PBMCs cultured from LL patients were reestablished after exogenous addition of exogenous IL-12/IL-18 and they also observed a diminished IL-18R expression. They assume that recombinant cytokines can activate

several transcription factors that induce IFN-γ synthesis.

Moura et al quantified cytokine (IFN-γ, TNF and IL-4) mRNA of skin biopsies from leprosy patients and in long-term culture of MB leprosy macrophages isolated from skin lesion and found that RNA expression in tuberculoid and MB lesions showed the profile expected of characteristic Th1 and Th2 response, respectively [62]. The inflammatory cells in all biopsies were successfully isolated. Although the number of cells varied between biopsies, it was highest in LL biopsies. In contrast to the biopsies TNF-α, IFN-γ and IL-10 expression in the tuberculoid and MB leprosy cells in 24-h culture and the cytokine levels in supernatants did not differ significantly. This study confirmed the feasibility of obtaining ex vivo macrophages from leprosy lesions and keeping them in long-term culture. This procedure may open new pathways to studying the interaction between *M. leprae* and human macrophages. A study showed that a panel of *M. leprae* recombinant protein for T-cell responses, measured by IFN-γ production, among leprosy patients[63]. After initial studies using PBMCs from leprosy patients, they transitioned their studies to simple whole-blood assay (WBA), which are more applicable in field or clinical settings. They demonstrated the utility of leprosy WBA that can be applied broadly in clinical or field settings.

The detection of hundreds of thousands of new cases of leprosy every year suggests that transmission of *M. leprae* infection still continues. Unfortunately, tools for identification of asymptomatic disease and/or early-stage *M. leprae* infection are lacking. Geluk et al reported that antigens with the most promising diagnostic potential were tested for their ability to induce cytokine secretion by using PBMCs from leprosy patients and controls in five different areas where leprosy is endemic[63]: 240 individuals from Brazil, Nepal, Bangladesh, Pakistan, and Ethiopia were analyzed for IFN-γ response to five recombinant proteins (ML1989, ML1990, ML2283, ML2346, and ML2567) and 22 synthetic peptides. Of these, the *M. leprae*-unique protein ML1989 was the most frequently recognized and ML2283 the most specific for *M. leprae* infection/exposure. Importantly, 50% of the healthy household contact and 59% of the controls in areas of endemicity had no detectable IgM antibodies to *M. leprae*-specific PGL-1. T-cell responses specific for leprosy patients and healthy household contacts

were observed for ML2283 and ML0126-derived peptides, indicating that *M. leprae* peptides hold potential as a rapid diagnostic tools for early detection of leprosy.

CYTOKINE PRODUCTION DURING LEPROSY REACTIONS

All types of leprosy reactions are believed to be immunologically mediated, but the mechanisms responsible for each type of reaction remain poorly understood. Jacob et al reported that leprosy reactions presented 71.4% patients with leprosy seen at their clinic[64]. They identified that leprosy reactions occur frequently among patients living in non-endemic area and may occur before the initiation if multidrug therapy (MDT), during MDT, or even years after completion of therapy and may produce significant neurologic sequela. Balagon et al observed 23 relapses, 6-16 years after MDT (mean, 10,5 years). Cumulative risk was 6.6%. The data suggest relapses are related to activation of dormant organisms (persisters) not killed by MDT rather than new infection[65].

Cytokine production during Type 1 (reversal) reactions

T1R are the result of spontaneous enhancement of cellular immunity and delayed hypersensitivity to *M. leprae* antigens. Measurement of soluble IL-2 receptor levels in patient sera found high levels in T1R when the patients first presented for treatment and found that these levels declined steadily during treatment[66]. During T1R, increases in expression of the genes for several proinflammatory cytokines, including IL-1, IL-2, IL-12, IFN-γ, and TNF-α, have now been documented in several studies[9,67]. This activation is present both locally, in reacting skin lesions, and systematically, in serum and in circulating leukocytes. The pattern of cytokine expression has suggested to many investigators that T1R represent a spontaneously enhanced Th-1 response. Clinical studies have determined that the serum levels of some of these cytokines decline during the course of successful prednisolone treatment of T1R but show little or no reduction during the treatment of ENL reactions. Such a decline has been documented, for example, for indicators of inflammation such as neopterin and iNOS[68], as well as for cytokines and receptors more indicative of immunologic function such as soluble

IL-2 receptor, IFN-γ, IL-6, IL-10, IL-12, and IL-13[66,68,69]. As previously described, during the clinical course of leprosy, a substantial proportion of patients develop reactional episodes (T1R, or RR, and type II, or ENL) of acute inflammation affecting skin and nerves[9,70]. The precipitating factors and pathophysiological mechanisms involved in triggering both types of reaction remain ill defined. Although the prevalence of leprosy is declining because of the broad coverage of MDT, a significant impact on the incidence and development of reactional episodes has not been achieved. Therefore, understanding mechanisms governing the induction of reactional episodes is likely to facilitate novel strategies to prevent or treat these clinical inflammatory complications, which occur during and even after MDT[21,65,71-73]. Proinflammatory cytokines (TNF-α, IFN-γ, and IL-1β) are known to be released in vivo and ex vivo during episodes of RR and ENL[9,67]. Convincing data indicate that TNF-α is likely to be a marker of tissue injury throughout the leprosy spectrum, mediating local and systemic inflammatory manifestations during reactional states (e.g., ENL, RR, and neuritis). Previous studies have demonstrated elevated concentrations of TNF-α in plasma during ENL and enhanced TNF-α secretion following in vitro stimulation of ENL patients' PBMCs[9,29]. Furthermore, Sampaio et al have observed that TNF-α release from PBMC cultures from ENL patients, following in vitro exposure to *M. leprae*, was far higher than that observed from highly purified monocytes prepared simultaneously from the same patient[19]. These data suggest that cognate interaction between mononuclear cells in PBMC from ENL patients is required for maximal TNF-α production in response to *M. leprae*. Such effects may be mediated through release of soluble factors and/or cell - cell contact.

Stefani et al explored the prognostic value of in situ cytokine patterns in 39 patients with single-skin-lesion PB leprosy before single dose therapy, with 3 years of follow-up[28]. IFN-γ, IL-12, IL 10, IL 4, TNF-α, and macrophage inflammatory protein (MIP)-1α mRNA was quantified in skin biopsy samples at diagnosis. They found that *M. leprae* DNA was detected in 51.4% of cases. Type 1 immunity predominance with measurable IFN γ and undetectable IL-4, which is indicative of effective CMI, is compatible with both the RR (33.3%) and the resolution of lesions (64.1%) observed. A positive correlation between IL-12 and IFN-γ indicated type 1

polarization via IL-12. Positive correlations between key regulatory cytokines IL-10 and IFN-γ, IL-10 and IL-12, and IL-10 and TNF-α suggests that there may be some level of an intralesional pro-or anti-inflammatory mechanism essential in avoiding immunopathology. Faber et al estimated serum levels of cytokines (IL-4, IL-5, IFN-γ, TNF-α), cytokine receptors (TNFR I and II) and one monokine (neopterin) in seven leprosy patients to establish disease associated for reversal reactions[74]. Sera were collected at diagnosis of leprosy, at the onset of reversal reactions and at different time points during and at the end of prednisone treatment of reactions. They found that six of the seven patients showed increased levels of neopterin either at the onset of reversal reaction or 1 month thereafter, and levels declined on prednisone treatment to that seen at the time of diagnosis without reactions. Surprisingly, no consistent disease associated cytokine profile was observed in these patients. Interestingly, serum TNF-α levers were increased in the same patients even after completion of prednisone treatment, indicating ongoing immune activity. These studies demonstrate that despite cytokines levels in leprosy serum being inconsistent in relation to reversal reaction, serum neopterin measurement appears to be an useful biomarker in monitoring RR patients during corticosteroid therapy. Belgaumkar et al observed that mean cytokine levels were significantly higher in the patients group as compared to the controls[33]. In the non reactional patient group, pure neuritic leprosy patients showed higher levels of IFN-γ which were directly proportional to the extent of nerve involvement. Lepromatous leprosy patients had the highest levels of IL-6. T1R and ENL reactional patients had higher levels of IFN-γ and IL-6, respectively as compared to nonreactional patients. These results suggest that pure neuritic leprosy and BT patients in T1R are at greatest risk for nerve and tissue damage. Thus, cytokines have the potential to play a significant role in classification, prognosis and treatment of leprosy.

Cytokine production during Type 2 leprosy reaction (erythema nodusum leprosum, ENL)

ENL occur in patients with poor cellular immunity to *M. leprae*, abundant bacilli (i.e.,antigen) in cutaneous and peripheral nerve lesions, and a strong polyclonal antibody response with high

levels of circulating immunoglobulins. Based primarily on histological evidence, Wemambu et al proposed that ENL represents an Arthus-like phenomenon mediated by immune complexes[75]. Immunoglobulin and complement deposition have been demonstrated in the skin lesions, and serum complement is decreased in these patients, consistent with this hypothesis, and some mycobacterial constituents have been identified in some of these complexes [76]. Other studies have identified possible evidence of cellular immune activation in ENL, including increases in circulating IFN-γ, TNF-α, and IL-12[77]. Increases in mRNA levels for these cytokines have also been observed in biopsies of skin lesions, indicating that cellular immune activation is occurring locally. In contrast, increases in the expression of IL-6, IL-8, and IL-10 mRNAs and sustained expression of IL-4 and IL-5 mRNAs, all cytokines associated with neutrophil chemotaxis, antibody production, and reduced CMI, were observed in ENL lesions. As described previously, ENL is an immune-mediated complication of leprosy presenting with inflammatory skin nodules and involvement of multiple organ system, often running a protracted course. Immune complex production and deposition as well as complement activation have long been regarded as the principal etiology of ENL. However, new data show that CMI is also important. Recently, Kahawita et al showed that ENL is characterized by an inflammatory infiltrate of neutrophils with vasculitis and/or panniculitis[11]. There is deposition of immune complexes and complement together with *M. leprae* antigens in the skin. In addition, they showed that the major T-cell subtype in ENL is the CD4 cells, in contrast to lepromatous leprosy where CD8 cells predominate. The cytokines TNF-α and IL-6 are consistently found while IL-4 is low or absent in ENL lesions, indicating a Th1 type response. Keratinocyte 1a and intercellular adhesion molecule-1 (ICAM-1) have been shown to be present in the epidermis in ENL, which is evidence of a cell-mediated immune response.

CHEMOKINE PRODUCTION IN PATIENTS WITH LEPROSY

In response to *M. leprae* antigens, mononuclear cells from patients with lepromatous leprosy failed to release monocyte-activating cytokines. In contrast, mononuclear cells from patients with tuberculoid leprosy secreted such cytokines, which activate monocytes

to inhibit the intracellular multiplication of *L. pneumophila*[78]. Kirkaldy et al have investigated the expression of chemokines and their receptors in leprosy skin lesion using skin biopsies from 25 patients across the leprosy spectrum. They found that expression of CC chemokine MCP-1 (macrophage inflammatory protein-1) and RANTES (regulated upon activation normal T cells expressed and secreted protein, a chemokine) were elevated significantly in BT leprosy in reversal reaction compared to non-reactional borderline tuberculoid leprosy, but there was no difference in expression of IL-8 chemokine[79]. Surprisingly, there was no significant difference in the expression of CC (CCR2 and CCR5) and CXC (CXCR2) chemokine receptors across the leprosy spectrum. The presence of a neutrophil chemoattractant IL-8 in leprosy lesions, which do not contain neutrophils, suggests strongly role of IL-8 as a monocyte and lymphocyte recruiter in leprosy lesions. These results suggest that the chemokines and their receptors, which are know to chemoattract T cell ad macrophages, are involved in assembling the cellular infiltrate found in lesions across the leprosy spectrum.

Diagnosis of leprosy is usually made clinically and there are no test available for the routine laboratory diagnosis of the disease. Recently, one study investigated the potential role of chemokines as biologic marker of disease activity[35]. The authors used an enzyme-linked immunosorbent assay to measure chemokines in plasma of patients with leprosy and non-infected individuals. They found that there were significantly greater concentrations of the chemokines CCL3 and CCL11 in plasma of leprosy patients than in non-infected individuals. In a group of selected individuals, CCL11 was useful in diagnosis of leprosy, thereby suggesting that measurement of this chemokine may be useful as an aid in diagnosing leprosis[35]. More recently, Sefani et al showed that potential biomarkers of T1R were CXCL10 and IL-6 whereas IL-7, PDGF-BB and IL-6 , may be laboratory markers of ENL[7].

EFFECT OF PREDNISOLONE AND/OR ANTI-LEPROSY DRUG TREATMENT ON CYTOKINE PRODUCTION IN DIFFERENT TYPE OF LEPROSY

MDT caused a reduction in serum cytokines (IL-2R, IL-10, IL-1β) correlated with a reduction in the bacterial

burden. It is advisable to continue MDT for PB patients for 1 year. Serum IL-1β levels may have prognostic value for the susceptibility of leprosy patients to the development of reactions[80]. Shin et al observed the dynamics of cytokine expression using RT-PCR, such as TGF-β, IL-10, and IFN-γ in the lesions taken from borderline lepromatous leprosy patients before and after MDT for 4 weeks[81]. They showed that before treatment, cytokines were expressed in order of IL-10, iNOS, TGF-β and IFM-γ. After 4 week treatment, cytokines were expressed in order of iNOS, IL-10, TGF-β1 and IFN-γ and the changes of cytokine expression after 4 week treatment were not significant. They suggest that TGF-β1 and IL-10 may contribute to immune suppresion in MB patients, and that TGF-β1 suppresses iNOS expression in macrophages.

Post-treatment lepromatous leprosy patients secreted relatively high levels of IL-10 response to *M. leprae*, whereas one self-cured tuberculoid leprosy patients produced simultaneously high levels of IFN-γ and TNF-α[73]. Recently, Atkinson et al demonstrated that the presence of IL-10 and IL-6 in the skin lesion of patients with T1R[69]. The patients were receiving standardized treatment for T1R: a reducing course of daily oral prednisolone for 6 months. Biopsies were taken before treatment and during treatment at weeks 1, 4, and 6 months. IL-13 was observed in the lesions of most patients. By week 4 of treatment, the presence of IL-13, IL-10, and IL-6 in the lesions had decreased significantly. Although some patients showed significant clinical skin sign improvement within one week of therapy, no concomitant decrease or increase in any of the cytokines was observed at this time point. Interestingly, some cytokine activity within the lesion was observed after 6 months of treatment[69].

Regulation of inflammation in leprosy may be influenced by local concentrations of active cortisol and inactive cortisone, whose concentration are regulated by enzyme in the cortisol-cortisone shuttle. Andersson et al investigated cortisol-cortisone shuttle enzyme in the skin of leprosy patients with T1R[71]. They found that gene expression of 11beta-hydroxysteroid dehydrgenase (11betaHSD) type 2, which converts cortisol to cortisone, is down-regulated in skin from T1R lesion. However, expression levels of 11beta-HSD type 1 which converts cortisone to cortisol, were similar in skin with and without reactions and did not change during anti-leprosy drug treatment. Prednisolone

treatment of patients with reactions is associated with an up-regulation of 11beta-HSD2 expression in skin. The down-regulation of 11beta HSD2 at the beginning of a reaction may be caused by pro-inflammatory cytokines in the leprosy reactional lesion and may be a local attempt to down-regulated inflammation. However, in leprosy reactions, this local response is insufficient and exogenous steroids are required to control inflammation[71].

Andersson et al investigate the effect of prednisolone treatment on TNF-α, IL-1β, IL-10 and transforming growth factor β1 (TGF-β1) mRNA expression in blood and skin biopsies from patients with T1R in India[82]. They observed that after 1 month of prednisolone treatment the sizes of the skin granuloma were reduced, as were the grades of cell positive for TNF-α and IL-10 in skin lesions. Increased production was seen in skin lesions after 6 months of prednisolone treatment. Expression of mRNA for TNF-α, IL-1β, and TGF-β 1 was reduced, whereas no change in IL-10 mRNA was detected during treatment. In addition, they observed the circulating cytokine profiles were similar in patients with and without T1R, and prednisolone treatment had no detectable effects on cytokine expression in the blood. Surprisingly, patients with improved skin and nerve function and patients with nonimproved skin and nerve function had similar cytokine profiles, suggesting that clinical improvement is no directly mediated by the cytokines studied. More recently, Iyer et al investigated the profiles of serum cytokines, such as IL-4, IL-6, IL-10, IFN-γ , TNF-α, the soluble IL-6 receptors (sIL-6R), soluble T cells (sCD27) and macrophage (neopterin) activation marker and *M. leprae*-specific anti-PGL-1 IgM antibodies in relation to the leprosy spectrum and reactions[21]. Follow-up serum samples after corticosteroid treatment were also tested for cytokine levels. Interestingly, they observed a wide variability in cytokine levels in the patient groups. However, IFN-γ and sIL-6R were elevated significantly in ENL patients compared to non-ENL patients. Furthermore, levels of IFN-γ, TNF-α and sIL-6R declined significantly upon corticosteroid treatment of ENL[21]. This study suggests that limited applicability of serial measurement of the cytokines IFN-γ TNF-α and sIL-6R in monitoring therapeutic efficacy of ENL patients. However, a cautious approach to interpreting serum cytokine profiles and a further search for a wide panel of more disease-specific marker is recommended in future studies. Single skin lesion, paucibacillary (SSL-PB) disease is

considered one of the earliest clinical forms of leprosy. Sousa et al recruited SSL-PB leprosy volunteers (N=135) in three Brazilian endemic regions, treated with single-dose rifampin, ofloxacin, and minocycline (ROM), were monitored for 3 years[72]. Poor outcome was defined as T1R with or without neuritis. Mitsuda and *M. leprae* DNA polymerase chain reaction (ML-PCR) were performed at baseline. The majority of volunteers were adult with a mean age of 30.5 years: 44.4% were ML-PCR positive. During follow-up, 14.8% of the patients had poor clinical outcome, classified as a type 1 reactions. It is worth mentioning that during the 3-year clinical follow-up after ROM therapy, no severe reactional episode was observed and the conventional steroid therapy was sufficient to prevent nerve damage[72]. Recetnly, in addition to corticosteroid, many other immunosuppressive agents on leprosy reactions are found to be thalidomide, methotrexate, cyclosporin, azathiprine, petoxifylline, and mucopenolate mofetil.

EXPERIMENTAL LEPROSY

The armadillo was the first animal model of leprosy[83]. In addition, The SCID (severe combined immunodeficient) mouse lacks both B and T cells that can mount an effective cellular and humoral immune response to microorganisms and other foreign antigens. SCID mouse infected with *M. leprae* produced IL-6, TNF-α, and IFN-γ[84]. A SCID mouse model of leprosy could be useful to investigate potential vaccine strategies using human cells in a context in which the growth of the organisms is monitored. Pena et al characterized the recombinant IFN-γ from the nine-banded armadillo and showed that response of armadillo macrophage to recombinant protein (rDnIFN-γ) is similar to that which occurs in human and demonstrated a potentially important value of the armadillo as a model in leprosy research[32].

The immune response of leprosy patients can be highly diverse, ranging from strong cellular responses accompanied by an apparent deficit of *M. leprae*-specific antibodies to strong humoral responses with a deficit of cell-mediated responses. Leprosy takes many years to manifest. Importantly, and in contrast to subcutaneous *M. leprae* footpad infection, systemic *M. leprae*-specific IFN-γ and antibody responses were detected following intradermal infection of mouse ear[85]. These results indicate the utility of intradermal mouse ear infection for both induction and

understanding of the immune response during *M. leprae* infection and the identification or testing new leprosy treatments. On infection with many intracellular bacteria, especially mycobacterial pathogens, a granulomatous response ensues. This complex process involves the participation of CMI, activation of endothelial cells, enhancement of adhesion molecule expression management of macrophage and lymphocyte infiltration and induction of the microbiostatic and microbicidal effect of macrophages[9]. Furthermore, extensive intercellular communication, chiefly through cytokine and chemokine signals, is required to orchestrate this cellular accumulation and results in a three-dimensional structure that limits or prevents dissemination of the pathogen and is largely protective. The contribution of both soluble and membrane bound TNF to granuloma development have been well established[9]. Lymphotoxin-α is also a member of the TNF superfamily, In experimental leprosy, both lymphotoxin-α and TNF are essential for the regulation of the granuloma, but they have distinct roles in the recruitment of lymphocytes and maintenance of the granulomatous response during chronic *M. leprae* infection[12].

Leprosy elimination has been a goal of the WHO for the past 15 years. Widespread BCG vaccination and MDT have dramatically reduced worldwide leprosy prevalence, but new case detection rates have remained relatively constant. These data suggest that additional control strategies, such as a subunit vaccine are required to block transmission and to improve leprosy control. Raman et al recently identified several *M. leprae* antigens that stimulate IFN-γ secretion upon incubation with blood from PB leprosy patients, a group who limit *M. leprae* growth and dissemination[86]. Furthermore, they demonstrated that *M. leprae*-specific mouse T-cell lines recognize several of these antigens, with the ML0276 protein stimulating the most IFN-γ secretion. In addition, they demonstrated that combining ML0276 with Toll-like receptor 4 (EM005) agonist during immunization induces Th1 responses that limit local inflammation upon experimental *M. leprae* infection. Interestingly, despite the potent Th1 response induced by this regiment, it could not provide protection in terms of limiting bacterial growth. They noted that EM005 is the most potent adjuvant for stimulating a Th1 response and indicate that while a subunit vaccine containing the ML0276 protein may be useful for the prevention of

immune pathology during leprosy, it will not control bacterial burden and is therefore unlikely to interrupt disease transmission[86].

The armadillo was the first animal model of leprosy. Its role in the transmission of leprosy remains controversial. The sooty mangabey model of leprosy led to the discovery that rhesus monkeys were more susceptible to leprosy when coinfected simian immunodieficiency virus, but that leprosy may play a protective role against acquired immunodeficiency syndrome (AIDS) mortality. Recently, molecular methods have been developed for leprosy and may help resolve the role of zoonosis in leprosy[83]. In short, the importance of the T lymphocytes in host resistance was revealed in experimental *M. leprae* infection of neonatally thymectomized or congenitally athymic (nude) mice and rats and experimental *M. leprae* infections in cytokine knockout (KO) mice, particularly mice with a defective gene for IFN-γ (GKO), have substantiated the immunological importance of these cytokines across the leprosy spectrum and revealed compensatory mechanisms of host resistance to *M. leprae*[12]. In addition, the nine-banded armadillo (*Dasypus novemcitus*) is the only immunologically intact animal that regularly develops fully disseminated *M. leprae* infections. Intravenous inoculation with 10^8 to 10^9 bacilli regularly results in 10,000-fold increase in the number of *M. leprae* over span of about 18 months, and armadillos have been the hosts of choice for in vivo propagation of *M. leprae* for more than 30 years. With high burdens of bacilli in their reticuloendothelial tissues, armadillo can yield gram quantities of *M. leprae*[12,87-90].

CONCLUSION AND A VIEW

Leprosy is a chronic infectious disease caused by the intracellular pathogen *Mycobacterium leprae*. Although *M. leprae* still cannot be cultivated axenically and specific-vaccine has yet been developed, the estimated global prevalence of leprosy has been greatly reduced as a result of the multidrug therapy (MDT) program advocated by World Health Organization and its implementation with help of governmental and non-governmental organizations. However, although the reported number of registered cases worldwide has declined in the last two decades and infections in

individuals is curable, the reported number of new cases registered each year has remained the same. Despite a spectacular decrease in global prevalence, the incidence appears not to have changed significantly, and in fact, leprosy is more common than expected. Global elimination of leprosy has not been accomplished and does not appear likely, probably due to a complex mixture of social, economic and biological factors that cannot be resolved in the laboratory alone.

Leprosy may be complicated by inflammatory reactions. During the clinical course of leprosy a substantial proportion of patients develop leprosy reactions (type 1 and type 2 reactions). However, leprosy remain poorly understood. Immunopathological studies have generally found that type 1 (reversal) reactions compared to an up-regulation of Th1 type immune responses, and that type 2 (erythema nodosum leprosum, ENL) correspond to an enhancement of Th2 type of response. These findings are not yet very satisfying, however, since there are several unresolved discrepancies in these associations, and no information thus far indicate that what triggers reactions, or why they affect some patients, but not others. In fact, the cause, mechanisms, and treatment of these reaction remain highly problematic. The different types of reactions appear to have different underlying immunologic mechanisms, but these are poorly understood in spite of a substantial body of detailed descriptive information, and the factors that initiate them are unknown.

Recent discovery of several new effector T cells lineage further energize the research on Th cells. Effector T cells are the key players in steering the immune response to execute immune functions. The differentiation of naive T cells into fully functional effector T cells is characterized by acquisition of new profile of cytokine production. Key advances in the understanding of effector T cells have been tied to cytokine biology. Cytokine production is vital for the classification and functions of effector T cells. Owing to their critical and diverse functions in controlling immune responses, the functions and regulation of cytokine have been a subject of intense investigations. Recently, several types of effector T cells have been discovered and have distinct biological functions. Th1, Th2, Th17, Th9 and follicular helper T (Thf) cells and regulatory T cells produce a variety of cytokines. They produce lymphokines, proinflammatory

cytokines, antiinflammatory cytokines, growth factors and chemokines. It is not surprising that abnormalities in cytokines, their receptors, and the signaling pathways that they initiate are involved in a wide variety of disease. Resistance to *M. leprae* mediated through cellular immune response and involves a complex interplay of cytokines and chemokines. In this Review, I discussed the roles of cytokines in leprosy, cytokine profiles in different types of leprosy reactions, and effect of anti-leprosy treatment on cytokine productions. In addition, I briefly outlined experimental leprosy and recent studies demonstrating cytokine gene expression change during type 1 and type 1 leprosy reactions. There are many unresolved questions concerning immunopathogenesis of leprosy and much work is needed in the years to come to reveal what effects different cytokines and their combinations have on the effector T cells functions and through what mechanism(s) in leprosy. There are still many unanswered questions. It will address both challenge and opportunities to establish the global goal to eliminate leprosy in this world through expansion of our understanding of immunopathology.

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